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PRINCIPAL INVESTIGATOR: Terry Van Dyke, Ph.D.

CONTRACTING ORGANIZATION: University of North Carolina
Chapel Hill, North Carolina 27599-7510

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Terry Van Dyke, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of North Carolina
Chapel Hill, North Carolina 27599-7510

E-MAIL:
tavandy@email.unc.edu

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The p53 and pRb tumor suppressor pathways are frequently altered in human breast cancer. Although animal models have begun to explore mechanisms for these proteins, the roles can be different depending on the cancer type. The mechanisms by which p53 and pRb suppress tumorigenesis in breast cancer remain unclear.

Our previous studies in a mouse brain epithelial tumor model have demonstrated the importance of pRb in tumor initiation and of p53 in tumor progression, and have also established p53-dependent apoptosis as a means of tumor suppression. In this model, brain cells are induced to proliferate aberrantly by tissue-specific expression of T_{121}, a modified T antigen oncoprotein that inactivates pRb. This causes slow-growing, but highly apoptotic tumors. Further inactivation of p53 causes a dramatic decline in cell teath and rapid acceleration of tumor growth.

Here, we propose similar studies to examine the pRb and p53 roles in breast cancer. The full T antigen oncoprotein (inactivates both pRb and p53) has been shown to induce mammary tumors in transgenic mice. Here the T_{121} oncoprotein will be tissue-specifically expressed in mammary epithelium by mammary-specific promoters to test the role of pRb. Further analysis using knock out strains will then address the role of p53 when both pRb and p53 are inactivated. Impacts of the pRb inactivation, and of coexisting pRb and p53 mutations on apoptosis, proliferation, morphology abnormalities, and neoplastic growth in mammary glands will be assessed. Such preclinical animal models are essential for progress in breast cancer research.

The approach proposed here is novel because the role of pRb has not previously been tested and the p53 tumor suppression mechanism in breast cancer is not yet understood. Moreover, we expect that this approach will provide us a preclinical mouse model(s) to study tumor progression (angiogenesis, invasiveness and metastasis) that accounts for high mortality of breast cancer.

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Terry Van Dyke 7/27/00

PI - Signature Date

Gayle Henry

Progress Report: July 1, 1999 – June 30, 2000

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Introduction

An understanding of how breast cancer develops and the design and testing of innovative therapies will require the establishment of preclinical animal models. This is because of the distinct changes in breast cells that lead to this cancer cause certain biological changes that arise within a very specific tissue environment. We are fortunate that technologies exist to introduce specific genetic changes into the experimental mouse. This allows us to determine the impact of genetic changes observed in human cancer in the context of the whole organism. The pRb tumor suppressor pathway is altered in a large fraction of human breast cancers. Yet, the impact of pRb inactivation on mammary tissue has not been tested before. We will determine the effect on mammary tissue after inactivation pRb proteins by tissue specific expression of viral oncoproteine, T_{121}. We will further test the role of the p53 tumor suppressor – a gene that is also altered in about 50% of human breast cancers. This will be don by breeding the mice with mammary-specific pRb inactivation to p53-deficient mice. Our preliminary work suggests that these experiments have every chance for success in producing a valued preclinical model of breast cancer.

Body

Original Specific aims

We have explored the function of the pRb and p53 tumor suppressor proteins in several sites within the animal using tissue-specific promoters to express wild-type and mutant T-Ags combined with the use of knock-out mice. Our studies have taught us that cell type dictates the role of tumor suppressor inactivation in tumorigenesis. In this grant, using the same approach, we proposed to determine whether the brain tumor model we previously described indicative of tumorigenesis mechanisms in mammary epithelium or whether distinct strategies are utilized.

Progress

The WAP-T_{121} expression vector has been made and introduced into the mice (SOW Task 1.a). We have produced several lines of TgWAP- T_{121} mice that develop consistent abnormalities in the mammary epithelium (SOW Task 1.b), and one line of TgWAP-T_{121} mice have developed mammary epithelial tumors. Initially, 7 founder mice were generated, of which 4 were propagated to establish lines (Table 1)(SOW Task 1.d and 1.e). Founder mice 1 and 8 and all F1 mice from founder 0 died suddenly at about 6 weeks of age. We are currently analyzing this unexpected phenotype. Line 7 appears to generate some mice with this phenotype as well, possibly due to segregation of multiple transgene insertions. Importantly, all of the established lines show mammary-specific expression of T_{121} (Fig. 1)(SOW Task 1.c and 2.a) and develop mammary abnormalities upon lactation when the WAP promoter is induced. These mice showed mammary gland abnormalities, which we have now characterized more fully.

In each line, high proliferative and apoptotic indexes within the T_{121} expressing epithelium are observed (Fig. 1 and 2)(SOW Task 2.e). In lines 6 and 7, which express the highest T_{121} levels, mammary glands are smaller than normal and these mice fail to nurse. Lines 2 and 3 are able to nurse and hyperplasia is present within the mammary

epithelium. The mosaic founder # 3 was particularly informative, in that the aberrant proliferation and apoptosis was associated only with T_{121} -expressing cells indicating a cell-intrinsic direct effect of the transgene and ruling out secondary effects on the cells due to lactation defects (Fig. 1, center panels)(SOW Tasks 2.a, 2.b, 2.c, 2.d). More importantly, in line 6, female mice developed mammary gland tumors around 13 months of age after 5 pregnancies. All animals will continue to be aged and assayed for tumors.

We have begun to generate TgWAP-T_{121} *p53*$^{+/-}$ and TgWAP-T_{121} *p53*$^{-/-}$ mice(SOW Task 3.a). The former have been bred to generate pregnancies for biopsis and to produce the latter genotype.

We will continue to breed to generate TgWAP-T_{121} *p53*$^{+/-}$ and TgWAP-T_{121} *p53*$^{-/-}$ mice and collect pregnancy and lactating mammary glands for analysis of apoptosis, proliferation, histological abnormalities and tumors. In comparison with TgWAP-T_{121}p53$^{+/+}$ mice, these data will provide us valuable information on the impact of inactivation of p53 and pRb on breast cancer.

Table 1. Summary of TgWAPT121 Transgenic Mice

Transgenic Line	Expression		Gross Phenotype Abnormalities	Mammary Gland
	Tissue	Protein		
TgWAPT121-0	N.D	N.D	death[2]	N.D
TgWAPT121-1	N.D	N.D	no line , death[3]	N.D
TgWAPT121-2	lactating MG	++	normal	HAP, HM, hyperplasia
TgWAPT121-3	lactating MG	+++	normal	HAP, HM, hyperplasia
TgWAPT121-6	lactating MG	++++	not nursing	HAP, HP, SL, HM, tumors
TgWAPT121-7	lactating MG, brain, kidney	++++	not nursing, death[1]	HAP, HP, SL, HM
TgWAPT121-8	N.D	N.D	no line, death[3]	N.D

(MG): mammary gland; (N.D): not determined; (HAP): high apoptotic bodies; (HP): high proliferation; (SL): small-sized mammary lobules; (HM): high mitotic figures.

[1]~50% mice died of unknown causes.

[2]All mice died at 1 to 1.5 month old.

[3]Founder died at 1 month old, no offspring.

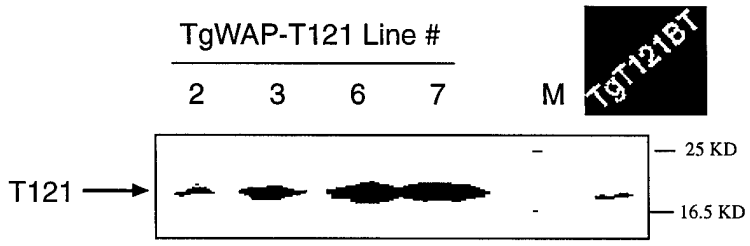

Figure 1. T121 expression in mammary glands of TgWAPT121 mice. Mammary gland lysates (100 μg) from pregnant mice (1st pregnancy) of indicated TgWAPT121 lines were analyzed by immunoblotting to detect T121 protein expression. Days from onset of lactation are: line 2, day 8; line 3, day 11; line 6, day 14; line 7, day 2. TgT121BT: positive control lysate from a brain tumor expressing T121.

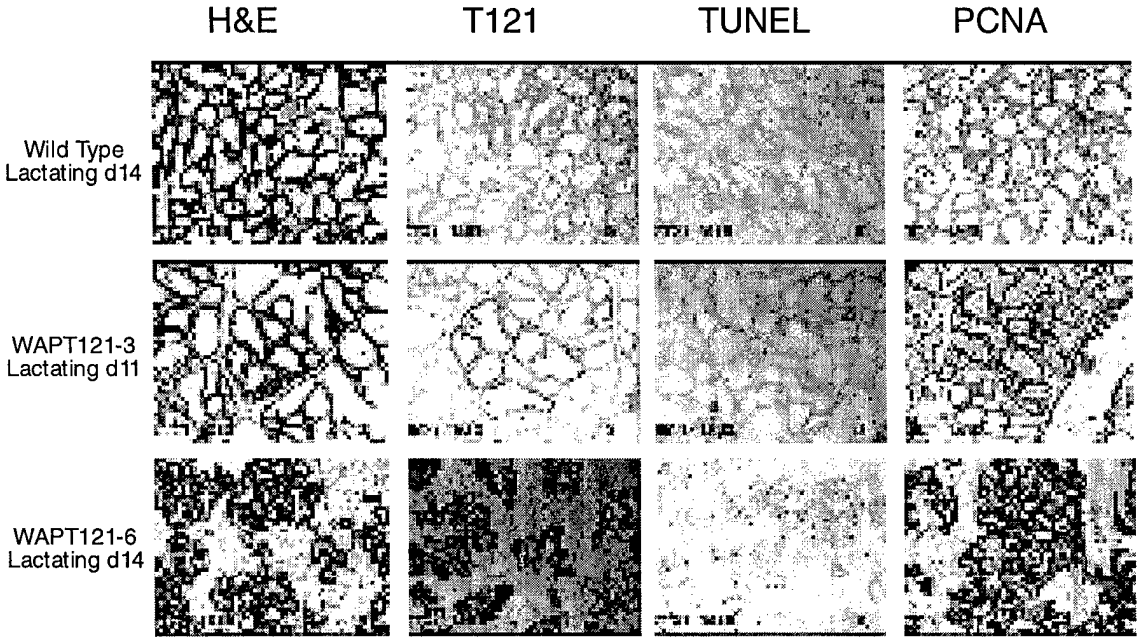

Figure 2

Key Research Accomplishments

1. We have generated TgME-T_{121} transgenic mice. They all have specific mammary gland expression of the transgene.
2. We have characterized TgME-T_{121} mice. In one of the transgenic lines, females have developed mammary gland tumors. We are monitoring survival and examining tumor types at terminal stage.
3. We are in the process of generating TgME-$T_{121}p53^{+/-}$ and TgME-$T_{121}p53^{-/-}$ mice.

Reportable Outcomes

None at this time.

Conclusions

None at this time.

References

None at this time.

Appendices

None at this time.